

The Impact of Suckling and Post-weaning Period on Blood Chemistry of Piglets

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Abstract

The aim of this study was to determine the impact of dietary changes during the suckling and post-weaning period on selected haematological, immunological and metabolic indices as well as the macro- and micro-mineral and antioxidant status of piglets. Twelve Large White piglets were kept according to the standards of pig rearing used in Slovakia until the end of the post-weaning period. Blood samples were taken from the piglets at the age of 30 days (the end of the suckling period) and 2 months (the end of the post-weaning period). At the end of the post-weaning period higher indices were found for RBC ($P < 0.01$), Hb ($P < 0.01$), WBC ($P < 0.05$), total Ig ($P < 0.05$), AST ($P < 0.01$), urea ($P < 0.01$), Se ($P < 0.05$), GSH-Px ($P < 0.001$), -SH groups ($P < 0.01$) and lower indices of albumin ($P < 0.05$), ALP ($P < 0.001$), LDH ($P < 0.01$), pancreatic amylase ($P < 0.01$), total lipids ($P < 0.01$), glucose ($P < 0.01$), total cholesterol ($P < 0.01$), bilirubin ($P < 0.001$), Ca ($P < 0.01$), SOD ($P < 0.01$), MDA ($P < 0.01$), vitamin A ($P < 0.05$), and vitamin E ($P < 0.05$) as compared to the end of suckling period. These results indicate that the dietary changes during suckling and post-weaning periods affected the majority of observed blood indices in piglets.

Nutrition, diets, immunoglobulins, haematology, blood indices

The characteristic features of the suckling period of piglets are an extremely high growth rate and rapid development of the organism (especially bones and parenchymatous organs) enabled by unique milk nutrition with a high fat content (Trávníček 1960) provided by the dam. Thus not only rapid growth but also deposition of fat in tissues of the body of piglets is possible. During the suckling period, piglets are routinely supplemented with concentrated creep feed (pre-starter) offered to them *ad libitum* from day 7 after birth. The piglets receive this diet until they reach the weaning age. Usually, a three-day weaning period is a transition phase from the milk nutrition to starter diets. During the post-weaning period, another complete feed for weaned piglets is fed. Gu and Li (2003) and Wu et al. (2007) reviewed the important role of fat and amino acids in swine nutrition and production, respectively. The feeding of complete diet for weaned piglets rich in nitrogen compounds and a balanced composition of essential nutrients enhances a remarkable increase of body mass via incorporation of amino acids mainly into the skeletal muscles. Therefore, the level of piglet nutrition plays an important role in their healthy growth and development that is very intensive during the first 2 months of their postnatal life.

The influence of nutrition during the suckling, weaning and post-weaning period of piglets on their immunocompetence (Klobasa et al. 1987; Salmon 1999), metabolism (Holub et al. 1978ab; Holub 1982; 1990; 1994), morphology of the gastro-intestinal tract (Van Beers-Schreurs 1996; Gu et al. 2002) and the activities of digestive enzymes in duodenum and jejunum (Makkink et al. 1994; Wattanakul et al. 2007) are well described. However, a complex knowledge of the concentrations of antioxidants and minerals as well as the activities of enzymes in the blood of piglets in these phases is limited.

Therefore, the aim of our study was to determine the effect of dietary changes during

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the suckling and post-weaning period on selected haematological, immunological, and metabolic indices as well as the macro- and micro-mineral and antioxidant status in piglets.

Materials and Methods

One litter of Large White piglets ($n = 12$), of average body mass (BM) of 1.6 kg, were treated with Ferridextran inj. (Spofa, CZ) and Selevit inj. (Biotika, SR) at the doses of 1.0 and 1.0 ml·kg⁻¹ of BM on the day of birth, respectively. The piglets were nursed by their dam for 32 days. The milk of Large White sows contains per 100g: total lipids 9.31 g; total cholesterol, 0.91 g; total proteins, 4.69 g; total solid, 5.77 g (Link et al. 2007), and per liter: Ca, 1.93 g; P, 1.15 g; Mg, 0.11 g; Na, 0.38 g; K, 0.72 g; Cu, 0.95 mg; Fe, 0.4 mg; Zn, 7.46 mg; Se, 0.016 mg. Csapo et al. (1995) obtained similar concentrations of macro- and micro-elements and found the following amounts of vitamin A and E in the sow milk: 0.92 ± 0.14 and 2.53 ± 0.23 mg·kg⁻¹, respectively.

At the age of 7 days, the nutrition was enriched with concentrated diet (OŠ-1) for sucking piglets (Tajba, SR) (Table 1).

Table 1. Composition of concentrated diet (OŠ-1) for sucking piglets fed from 7th day up to the age of 30 days

Component	%	Component	%
Corn	37	Methionine	0.1
Soybean extracted ground meal	24	Threonine	0.2
Barley	10	Ca(H ₂ PO ₄) ₂ · H ₂ O	0.7
Wheat	5	CaCO ₃	1.8
Wheat bran	7	NaCl	0.45
Wheat ground	6	Vitamin-mineral premix	0.03
Whey powder	10	Kenzyme WP Dry New ^a	0.05
Lysine	0.4		

OŠ-1 contained per 1 kg of DM: vitamin A, 8000 U; vitamin D₃, 1000 U; NC, 180 g; DF, 30-40 g; lysine, 11.5 g; treonine, 5.5 g; methionine and cysteine, 6.3 g; Ca, 8.0 g; P, 6.7 g; Na, 2.0 g; vitamin E, 20 mg; vitamin B₂, 3 mg; choline, 600 mg; Fe, 125 mg; Zn, 100 mg; Cu, 10 mg; Se, 0.1 mg; vitamin B₁₂, 20 µg
a-supplied per kg of diet: α amylase, 200 U; endo-1,3,(4)-β glucanase, 1175 U; endo-1,4-β glucanase, 2000 U; endo-1,4-β xylanase, 1000 U; bacilolisin, 225 U; 6-fytase, 499.5 FYT

The weaning of piglets was performed between days 30 and 32 after birth by reducing the sucking from 3-times to 2-times to once a day, respectively. During these three days the dietary supplement contained both OŠ-1 and OŠ-2 diets for weaned pigs (Tajba, SR), mixed at a ratio 2:1, 1:1 and finally 1:2, respectively.

During the post-weaning period (i.e. from day 32 until the age of 2 months), the piglets were fed the diet OŠ-2 (Table 2).

Table 2. Composition of concentrated feed (OŠ-2) for weaned piglets fed from 32nd day up to the age of 2 months before blood sampling

Component	%	Component	%
Corn	42.6	Lysine	0.2
Soybean extracted ground meal	23.7	Threonine	0.1
Barley	10	Ca(H ₂ PO ₄) ₂ · H ₂ O	0.7
Wheat	5	CaCO ₃	2.0
Wheat bran	5	NaCl	0.3
Wheat ground	5	Vitamin-mineral premix	0.03
Whey powder	5	Kenzyme WP Dry New ^a	0.1

OŠ-2 contained per 1 kg of DM: vitamin A, 8000 U; vitamin D₃, 1000 U; lysine, 11.5 g; treonine, 7.5 g; methionine and cysteine, 6.3 g; Ca, 7.0 g; P, 5.8 g; Na, 1.5 g; vitamin E, 20 mg; vitamin B₂, 3 mg; choline, 600 mg; Fe, 125 mg; Zn, 100 mg; Cu, 10 mg; Se, 0.1 mg; vitamin B₁₂, 20 µg
a-supplied per kg of diet: α amylase, 200 U; endo-1,3,(4)-β glucanase, 1175 U; endo-1,4-β glucanase, 2000 U; endo-1,4-β xylanase, 1000 U; bacilolisin, 225 U; 6-fytase, 499.5 FYT

The piglets were placed in a large pen with a fibre-board, and had free access to the pen with their dam only during the suckling period. All piglets had free access to water and creep feed. The room temperature of 24 °C

was kept during the whole experiment but the pen with piglets was heated with a lamp at the temperature of 32 °C until weaning.

At the age of 30 days, the piglets were weighed (average BM of 8.2 kg) and blood was collected from sinus ophthalmicus (Kováč et al. 1990) into heparinized tubes and centrifuged for blood plasma at 1180 g for 15 min and allowed to clot to obtain blood serum. The samples of blood, plasma and serum were immediately frozen and stored at -24 °C. The same procedure was performed at the age of 60 days (at the end of the post-weaning period) when the average body mass was 15.9 kg per animal.

The standard kits from Randox, UK were used to determine the activities of AST, ALT, ATP, γ GT, LDH, pancreatic amylase in serum and concentration of glucose in blood as well as measurement of total proteins, albumin, creatine, urea, total cholesterol, total lipids, bilirubin in serum and phosphorus in milk and serum by spectrometer Alizé (Lisabio, Fr.). The concentrations of Na, K, Ca, Mg, Cu, Fe and Zn in milk, concentrated feed and serum were analysed by flame AAS method (AAnalyst 100, Perkin-Elmer). The concentration of selenium in milk, concentrated feed and blood was measured using the fluorimetric method of Rodriguez et al. (1994). The indices of blood picture were assessed by the animal blood counter (ABC VET 16p, Trigon s.r.o.) and total immunoglobulins in serum by Spekol 211 (Carl-Zeiss Jena). Activity of blood glutathione peroxidase (GSHPx, EC 1.11.1.9) was determined using the Ransel kit (Randox, UK). Concentration of malondialdehyde in plasma was measured with the modified fluorimetric method according to Jo and Ahn (1998). Superoxide dismutase (SOD, EC 1.15.1.1) activity (Arthur and Boyne 1985) in erythrocytes was analyzed using kits from Randox, UK. Ellman's method (1958) was used to determine the concentration of sulphhydryl groups in plasma. The concentrations of vitamin A and E were assessed using HPLC technique according to Tučková and Kaštel (1999).

The experiment was carried out in accordance with established standards for animal care and use. The protocol was approved by local Ethics Committee.

Statistical analysis of all results was done using paired Student's *t*-test.

Results and Discussion

At the time of weaning, the immune system of piglets is not fully mature (Hampl et al. 1980; Trebichavský et al. 1988; Kovářů et al. 2002; Brown et al. 2006) and the immunity is still developing by contact of immune cells with viruses and bacteria naturally present in the environment. The indices of white blood cells (WBC), total immunoglobulins and albumin revealed significant differences in young pigs of two months of age (the end of the post-weaning period) compared to one-month-old piglets (the end of the suckling period). The WBC count showed an increasing tendency during the first two months of

Table 3. Haematological and immunological indices of piglets

Indices	End of suckling period (day 30 after birth)	End of post-weaning period (day 60 after birth)
RBC ($T \cdot l^{-1}$)	6.188 \pm 0.407	7.14 \pm 0.282**
Hb ($g \cdot l^{-1}$)	99.4 \pm 11.4	119.6 \pm 14.08**
MCV ($f l^{-1}$)	50.8 \pm 5.514	53.14 \pm 1.676
MCH ($f mol^{-1}$)	16.12 \pm 1.847	16.73 \pm 0.472
MCHC ($mmol \cdot l^{-1}$)	31.72 \pm 0.691	31.51 \pm 0.302
Tc ($G \cdot l^{-1}$)	710.6 \pm 186	701.0 \pm 104.9
WBC ($G \cdot l^{-1}$)	14.84 \pm 3.555	17.47 \pm 2.692*
Albumin ($g \cdot l^{-1}$)	39.29 \pm 3.428	36.17 \pm 1.545*
Total Ig (U ZST)	24.57 \pm 2.487	27.66 \pm 2.821*

Results are presented as mean \pm SD, n = 12; **P* < 0.05; ***P* < 0.01

Symbols: red blood cell (RBC); haemoglobin (Hb); mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values; mean corpuscular haemoglobin concentration (MCHC); thrombocytes (Tc); white blood cell (WBC); total immunoglobulins (Total Ig), units of zinc-sulphate test (U ZST)

postnatal life (Table 3), similar to findings of Zelníčková et al. (2006). Similarly, the erythrocyte numbers and haemoglobin content differed depending on the age of animals (Table 3). Our explanation of these changes is based on the fact that in the early postnatal ontogeny the extremely rapid development of organs is responsible for the formation and maturation of RBC. Moreover, the life span of erythrocytes is above 120 days. Egeli et al. (1998) found the physiological Hb amount to be up to 80 $g \cdot l^{-1}$; this indicates that the piglets in our experiment did not suffer from anaemia.

The activities of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and pancreatic amylase in serum reflect the growth of bones, production of energy and metabolism of

Table 4. Activities of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (γ GT), lactate dehydrogenase (LDH) and pancreatic amylase (μ kat \cdot l $^{-1}$) in blood serum of piglets

Indices	End of suckling period (day 30 after birth)	End of post-weaning period (day 60 after birth)
AST	0.414 \pm 0.069	0.590 \pm 0.101**
ALT	0.669 \pm 0.204	0.834 \pm 0.148
ALP	17.78 \pm 3.241	5.416 \pm 1.166***
γ GT	0.488 \pm 0.266	0.34 \pm 0.069
LDH	24.83 \pm 2.32	18.4 \pm 2.964**
Pancreatic amylase	44.48 \pm 6.93	37.51 \pm 5.73**

Results are presented as mean \pm SD, n = 12; ** P < 0.01; *** P < 0.001

the end of the suckling period. Tang et al. (1999) found a reduction of the specific ALP activities in duodenum and jejunum during the weaning of pigs.

The milk of sows contains high amounts of fat and carbohydrates (Trávníček 1960) that surpass those of the complete feed rich in nitrogen compounds and given during the post-weaning period. Total lipids, glucose, total cholesterol, bilirubin and urea are primarily affected by the nutritional value of the feed (Lauridsen and Jensen 2007; Trujillo-Coutino et al. 2007). Significant differences found in these indices in our experimental piglets confirm this view (Table 5). Although there were age-dependent

Table 5. Total proteins, total lipids (g \cdot l $^{-1}$), urea, glucose, total cholesterol (mmol \cdot l $^{-1}$), creatine and bilirubin (μ mol \cdot l $^{-1}$) in blood serum of piglets

Indices	End of suckling period (day 30 after birth)	End of post-weaning period (day 60 after birth)
Total proteins	65.39 \pm 3.802	62.91 \pm 3.778
Total lipids	6.379 \pm 1.863	3.521 \pm 0.624**
Urea	2.55 \pm 0.548	3.99 \pm 0.814**
Glucose	7.30 \pm 0.837	5.264 \pm 0.674**
Total cholesterol	4.730 \pm 1.324	2.721 \pm 0.636**
Creatine	93.61 \pm 16.64	98.54 \pm 18.18
Bilirubin	6.230 \pm 1.665	2.439 \pm 0.593***

Results are presented as mean \pm SD, n = 12; ** P < 0.01; *** P < 0.001

Table 6. Concentrations of P, Na, K, Mg, Ca (mmol \cdot l $^{-1}$) Fe, Zn, Cu (μ mol \cdot l $^{-1}$) in serum and Se (μ mol \cdot l $^{-1}$) in blood of piglets

Indices	End of suckling period (day 30 after birth)	End of post-weaning period (day 60 after birth)
Phosphorus	2.918 \pm 0.241	2.797 \pm 0.122
Sodium	138.3 \pm 1.767	138.7 \pm 2.36
Potassium	6.26 \pm 0.749	6.757 \pm 1.069
Magnesium	1.058 \pm 0.099	1.087 \pm 0.157
Calcium	3.04 \pm 0.239	2.676 \pm 0.21**
Iron	15.3 \pm 3.32	17.53 \pm 3.746
Zinc	15.03 \pm 3.397	13.271 \pm 2.997
Copper	26.2 \pm 3.914	24.39 \pm 3.384
Selenium	0.526 \pm 0.171	0.874 \pm 0.177*

Results are presented as mean \pm SD, n = 12; * P < 0.05; ** P < 0.01

carbohydrates, respectively. All these processes are extremely efficient during the suckling period. The significantly higher activities of ALP and pancreatic amylase in the serum of piglets at the end of the suckling period versus the end of the post-weaning period (Table 4), corroborate this phenomenon. On the other hand, the activity of aspartate amino transferase was found to be significantly lower in serum of pigs at the end of the suckling period. Tang et al. (1999) found a reduction of the specific ALP activities in duodenum and jejunum during the weaning of pigs. The milk of sows contains high amounts of fat and carbohydrates (Trávníček 1960) that surpass those of the complete feed rich in nitrogen compounds and given during the post-weaning period. Total lipids, glucose, total cholesterol, bilirubin and urea are primarily affected by the nutritional value of the feed (Lauridsen and Jensen 2007; Trujillo-Coutino et al. 2007). Significant differences found in these indices in our experimental piglets confirm this view (Table 5). Although there were age-dependent changes in the concentrations of the individual amino acids in the blood of piglets observed (Baranyiová et al. 1990), our results did not reveal a significant difference in the amount of total proteins.

During the whole suckling period, the sow's milk was the main source of nutrition for piglets, but the concentrated feed for sucking piglets was readily eaten and its consumption increased. The concentration of macro- and micro-elements in the blood serum depends on the nutritional composition of diet, age and physiological state of the animal. The concentrations of Na, K, Mg, Fe, Zn, Cu in the serum were similar, whereas the concentration of calcium in the serum and selenium in the blood reflected the disproportion between their amount in the feed given to piglets during both periods as well as their physiological requirements

for healthy growth (Table 6). Similarly, Egeli et al. (1998) found the concentration of minerals in the blood of piglets unaffected by their age.

Especially micro-elements are considered as antioxidant nutrients, Fe as a part of catalase, Cu and Zn as a part of superoxide dismutase (SOD) and Se as a part of glutathione peroxidase (GSH-Px) and all play interdependent roles in the antioxidant protection. The concentration of Se in the diets fed during the suckling period (milk; 0.016 mg·l⁻¹ with a supplement of OŠ-1; 0.1 mg·kg⁻¹ of DM) and the post-weaning period (concentrated feed for weaned pigs; 0.18 mg·kg⁻¹ of DM), resulted in significant changes in our observation period (Table 6 and 7). As shown also by other authors, a higher intake of Se from the diet is reflected in an elevated concentration of Se as well as the activity of GSH-Px in blood

Table 7. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in blood (U·g⁻¹ of Hb), concentrations of malondialdehyde (MDA), sulphhydryl (-SH) groups, vitamin A and E in blood plasma of piglets.

Indices	End of suckling period (day 30 after birth)	End of post-weaning period (day 60 after birth)
SOD	2790 ± 454.3	1959 ± 328.9**
GSH-Px	92.92 ± 11.77	156.2 ± 19.05***
MDA (μmol·l ⁻¹)	0.576 ± 0.154	0.36 ± 0.055**
-SH groups (mmol·l ⁻¹)	0.203 ± 0.066	0.406 ± 0.044**
Vit. A (μmol·l ⁻¹)	1.25 ± 0.146	0.878 ± 0.136*
Vit. E (μmol·l ⁻¹)	1.86 ± 0.272	1.32 ± 0.135*

Results are presented as mean ± SD, n = 12; *P < 0.05; **P < 0.01; ***P < 0.001

of animals (Gu et al. 1998; Lei et al. 1998; Boldizarova et al. 2005; Travnicek et al. 2008). The sensitivity of measuring SOD, GSH-Px and malondialdehyde (MDA) has made these indices a first choice for screening and monitoring the oxidative stress. Meanwhile, the concentration of vitamins and sulphhydryl groups is used for the assessment of antioxidant defence capacity of the body. The first phase in the postnatal life of an animal, i.e. the suckling period, is accompanied by an elevated generation of free radicals originating from intensive metabolic processes in cell compartments (Žitňanová et al. 2007). Superoxide anions are a specific substrate for dismutation catalyzed by SOD yielding hydrogen peroxides. The significantly increased SOD activity in erythrocytes of our suckling piglets (Table 7) seems to be associated with the aggravated formation of superoxide anions. These radicals are indicators of uncontrolled oxidation processes that primarily affect lipids, causing their peroxidation, i.e. the chain reaction generating lipoperoxide free radicals from unsaturated fatty acids, which can induce further damage of proteins and DNA (Kelly et al. 1998). The milk of sow contains high amount of lipids that after digestion form a potent source of primary targets for free radicals in the blood plasma of piglets. This fact was confirmed by a higher concentration of MDA and a lower concentration of sulphhydryl groups in blood plasma of suckling piglets in our study. A negative correlation was found between the concentrations of MDA and sulphhydryl groups in blood plasma (Calabrese et al. 1996; Mimic-Oka et al. 2005). On the other hand, the concentration of lipophilic vitamins in blood plasma could be affected by their different dietary intake as well as their ability to participate in the antioxidant defence of organism by free radical scavenging (Table 7).

In conclusion, the dietary changes during the suckling and post-weaning period in piglets have a significant influence on the majority of blood indices under study.

Vplyv prechodu z mliečnej výživy na príjem koncentrovaného krmiva na krvné indexy u prasiatok

Cieľom pokusu bolo sledovať hematologické, imunologické a metabolické indexy, ako aj makro-, mikro-minerálny a antioxidantný profil v krvi prasiatok (n = 12) Bielej Ušľachtilej ošípanej na konci mliečnej výživy (v 30 dni života) a po následnom 30 dňovom príjme koncentrovanej diéty (v 60 dni života). U 60 dňových prasiatok sme zistili štatisticky

zvýšené indexy: červených krviniek ($P < 0,01$), Hb ($P < 0,01$), bielych krviniek ($P < 0,05$), celkového Ig ($P < 0,05$), AST ($P < 0,01$), močoviny ($P < 0,01$), Se ($P < 0,05$), GSH-Px ($P < 0,001$), -SH skupín ($P < 0,01$) a významne znížené indexy: albumínu ($P < 0,05$), ALP ($P < 0,001$), LDH ($P < 0,01$), pankreatickej amylázy ($P < 0,01$), celkových lipidov ($P < 0,01$), glukózy ($P < 0,01$), celkového cholesterolu ($P < 0,01$), bilirubínu ($P < 0,001$), Ca ($P < 0,01$), SOD ($P < 0,01$), MDA ($P < 0,01$), vitamínu A ($P < 0,05$), and vitamínu E ($P < 0,05$) v porovnaní s hodnotami indexov stanovenými na konci mliečnej výživy. Záverom môžeme konštatovať, že zmena mliečnej výživy na príjem koncentrovaného krmiva významne ovplyvnila väčšinu sledovaných krvných indexov u prasiatok.

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